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SHORT REPORT

Increased soluble P-selectin levels following deep venous thrombosis: cause or effect?

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Summary. Deep vein thrombosis (DVT) is associated with coagulation abnormalities, but evidence of excess platelet activity is scant. Soluble P-selectin is a marker of platelet activity, with high levels being found in patients with thrombotic disease. We measured soluble P-selectin by enzyme-linked immunosorbent assay (ELISA) in plasma from 89 patients with objectively confirmed DVT and in 126 healthy age- and sex-matched control subjects, and found higher levels in the patients ($P = 0{\cdot}011$). Taking the risk of DVT with a level of soluble P-selectin < 238 ng/ml to be 1, the relative risk of DVT with a soluble P-selectin level >238 ng/ml was 2·1 (95% CI 1·2–3·6). These high levels may be a reflection of a generalized hypercoagulable state that, with factors such as the presence of persistent thrombin generation, could be responsible for excess platelet activation.

Keywords: soluble P-selectin, deep vein thrombosis, platelets.

Deep vein thrombosis (DVT) is a common problem, with a frequency of 1–3 per 1000 of the population each year (Rosendaal, 1997). An important role of the clotting system in the aetiology of thrombosis follows from the increased risk associated with abnormalities in the system, both in the anticoagulant (deficiencies in protein C, protein S and factor V Leiden) and in the procoagulant (high levels of factor VIII and factor II) systems. The beneficial prophylactic effect of anticoagulants such as heparin supports this concept. Despite this, there is relatively little evidence suggestive of a role for excessive platelet activity in DVT (van Hulsteijn *et al*, 1982). Nevertheless, an effect, albeit smaller than that for heparin, has been shown for antiplatelet agents in the prophylaxis of thrombosis, suggesting a role for platelet activity in its aetiology (Research Committee of the British Thoracic Society, 1992).

Adhesion molecules regulate leucocyte migration from the circulation during inflammation and in other conditions. One such molecule, P-selectin, is a membrane component of the platelet alpha granule and endothelial cell Wiebel–Palade body. A soluble form of P-selectin is present in the plasma, and it has been suggested that increased levels are representative of platelet activation (Jilma *et al*, 1996; Blann & Lip, 1997; Fijnheer *et al*, 1997). High levels have been described in diseases such as diabetes, atherosclerosis and thrombotic consumptive platelet disorders, and fail to correlate with platelet count (Chong *et al*, 1994; Ikeda *et al*, 1994; Jilma *et al*, 1996).

We therefore hypothesized that increased levels of soluble P-selectin would be present in the blood of patients who have had a DVT. As soluble P-selectin is influenced by smoking (Blann *et al*, 1997), we included this factor in our analysis.

MATERIALS AND METHODS

We tested our hypothesis by measuring soluble P-selectin in the plasma of a subgroup of the 474 participants in the Leiden Thrombophilia Study, who were recruited from three thrombosis services in The Netherlands. Exclusion criteria were malignancy, age less than 70 years and recent (< 3 months) use of coumarin derivatives. Each patient was matched to a healthy community-based subject of the same sex within 5 years of age. Venesection was performed a median of 18 months (range 6–48 months) after their objectively proven DVT. Full details of this study are available elsewhere (Koster *et al*, 1995; van der Meer *et al*, 1997).

Venous blood was obtained from an antecubital vein, and citrated plasma was collected after centrifugation at 2000 *g*

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for 10 min at room temperature and then stored at −70°C. Plasma was shipped to the UK in dry ice. All the plasma samples had been thawed and refrozen for another project. Thus, plasmas were thawed a second time and tested in large batches for soluble P-selectin by commercial enzyme-linked immunosorbent assay (ELISA; Takara-Shuzo, Japan). The intra-assay coefficient of variation of this assay was < 5%; interassay variance was < 10%.

We hypothesized that soluble P-selectin would be increased by about half a standard deviation (i.e. from 200 ng/ml in control subjects to 235 ng/ml in the patients, being 17·5%). Thus, for a power of 90% (i.e. 1-beta) to define an alpha of $2p < 0{\cdot}05$, we would need data from a minimum of 200 subjects. Thus, from the 602 samples of available plasma, 215 were selected for analyst-blind measurement of soluble P-selectin. Results were returned to The Netherlands for code breaking, which revealed that results had been obtained from 89 cases and 126 controls. Data were analysed for odds ratio, *t*-test or the chi-squared test, as appropriate, and is presented as mean and 95% confidence interval (CI) or percentage incidence.

RESULTS

Mean soluble P-selectin was 288 (95% CI 257–319) ng/ml in the 89 patients (39% men, mean age 46 years) with a DVT and was 242 (227–258) ng/ml in the 126 controls (41% men, mean age 46 years), an increase in sP-selectin of 19% ($P = 0{\cdot}011$) (Fig 1). The difference in soluble P-selectin between the groups was 46 ng/ml (95% CI of difference 30–72 ng/ml). Overall, 35 patients and 73 controls had a level of sP-selectin lower than the median level of the entire group (238 ng/ml), whereas 54 (= 61%) patients and 53 (= 42%) controls had levels above this figure [a difference of 19% (95% CI 6–32%), chi-square 6·81, $P = 0{\cdot}009$]. Taking the risk of DVT with a level of soluble P-selectin < 238 ng/ml as the reference, the excess relative risk (odds ratio) associated with a soluble P-selectin level > 238 ng/ml was 2·1 (95% CI 1·2–3·6).

The group of 215 subjects included 63 current smokers, 22 (= 25%) patients and 41 (= 32·5%) controls ($P = 0{\cdot}215$, chi-squared test). Mean soluble P-selectin was 276 (95% CI 249–303) ng/ml in the 63 smokers and 255 (233–277) ng/ml in the 152 non-smokers ($P = 0{\cdot}028$).

In a multiple regression analysis with soluble P-selectin as the dependent variable, the effect of group (i.e. patient or control) retained significance ($P = 0{\cdot}004$), but smoking lost significance ($P = 0{\cdot}157$). A multivariate model (logistic regression) with age, sex and smoking as covariates led to similar risk estimates.

DISCUSSION

Our study found increased levels of soluble P-selectin following DVT and confirms our previous observation of the influence of smoking on soluble P-selectin (Blann *et al*, 1997), although this effect is weak. The reasons for higher levels than we expected (about 200–220 ng/ml) are unclear but may relate to plasma preparation, such as a delay between venepuncture and centrifugation, or centrifugation for only 10 min at room temperature. However, whatever the reason, it would have been constant between cases and controls.

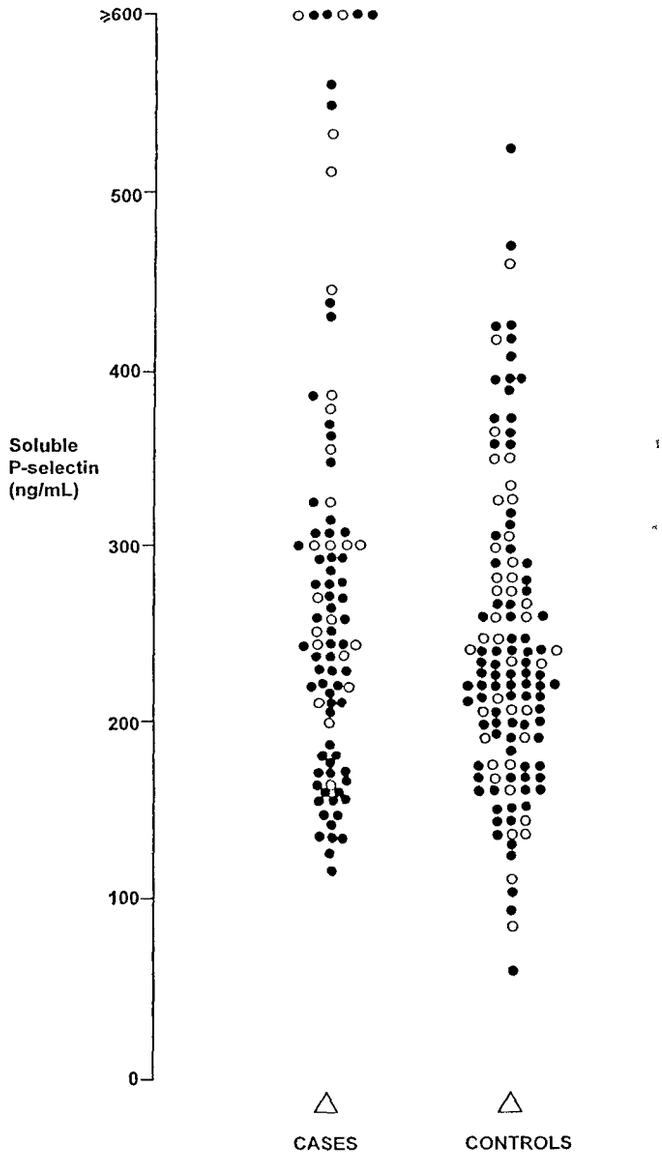

Fig 1. Levels of soluble P-selectin in the plasma of cases and controls. Open circles, smokers: closed circles, non-smokers.

Membrane-bound P-selectin is found at the surface of activated platelets and activated endothelial cells. Lack of correlation with endothelial marker von Willebrand factor is an example of a growing body of evidence that supports the hypothesis that increased levels of soluble P-selectin result from platelet activation (Jilma *et al*, 1996; Blann *et al*, 1997; Fijnheer *et al*, 1997; Kawabata *et al*, 1998). Raised soluble P-selectin in subjects who have suffered a DVT may indicate excessive platelet activation, supporting other data reporting raised beta thromboglobulin measured a few hours to 3 days

after a proven DVT (van Hulsteijn *et al*, 1982) Unlike beta-thromboglobulin, raised levels of soluble P-selectin are not caused by *ex vivo* platelet activation, nor do they correlate with platelet count (Chong *et al*, 1994, Jilma *et al*, 1996). However, as we took blood at least 6 months after the DVT and 3 months after the discontinuation of oral anticoagulation, we cannot discount the possibility that raised soluble P-selectin results from another difference between the patients and the controls For example, soluble P-selectin may be linked to another coagulation factor, or may be associated with post-thrombotic phenomena. Indeed, raised soluble P-selectin may be the result of platelet activation by thrombin, its fractions or other factors leading to a hypercoagulable state, as indicated by changes in markers such as fibrinopeptide A, prothrombin 1 + 2 or thrombin–antithrombin II complex

Although we found raised soluble P-selectin in the group of subjects who suffered a DVT, the wide scatter of data and poor discrimination implied that this marker has little value at the individual level. Nevertheless, it provides an indication that excess platelet activation may be related to the development, or a consequence, of DVT.

REFERENCES

Blann, A D & Lip, G Y H (1997) Hypothesis is soluble P-selectin a new marker of platelet activation? *Atherosclerosis*, **128**, 135–138

Blann A D, Steele, C & McCollum, C N (1997) Influence of smoking on soluble adhesion molecules and endothelial cell markers *Thrombosis Research*, **85**, 433–438

Chong, B H., Murray, B, Berndt, M C, Dunlop, L C, Brighton, T & Chesterman, C N (1994) Plasma P selectin is increased in thrombotic consumptive platelet disorders *Blood*, **83**, 1535–1541

Fijnheer, R, Frijns, C J M, Korteweg, J, Rommes H, Peters, J H, Sixma, J J & Nieuwenhuis, H K (1997) The origin of P-selectin as a circulating plasma protein *Thrombosis and Haemostasis*, **77**, 1081–1085

van Hulsteijn, H, Briet, E, Koch, C, Hermans, J & Bertina, R (1982) Diagnostic value of fibrinopeptide A and beta thromboglobulin in acute deep venous thrombosis and pulmonary embolism *Acta Medica Scandinavica*, **211**, 323–330

Ikeda H, Nakayama, H, Oda, T, Kuwano, K, Murashi, A, Sugi, K, Koga Y & Toshima, H (1994) Soluble form of P-selectin in patients with acute myocardial infarction *Coronary Artery Disease*, **5**, 515–518

Jilma, B, Fasching, P, Ruthner, C, Rumplmayr, A, Ruzicka, S, Kapiotis, S, Wagner, O F & Eichler, H G (1996) Elevated circulating P-selectin in insulin dependent diabetes mellitus *Thrombosis and Haemostasis*, **76**, 328–332

Kawabata, K, Nagake, Y, Shikata, K, Fukuda, S, Nakazano, H, Takahashi, M, Ichikawa, H & Makino, H (1998) Soluble P-selectin is released from activated platelets *in vivo* during haemodialysis *Nephron*, **78**, 148–155

Koster, T, Rosendaal, F R, Briet, E, van der Meer, F J M, Colly, L P, Trienseken, P H, Poort, S R, Reitsma, P H & Vandenbroucke, J P (1995) Protein C deficiency in a controlled series of unselected outpatients an infrequent but clear risk factor for venous thrombosis (Leiden Thrombophilia Study) *Blood*, **85**, 2756–2761

van der Meer, F J M Koster T, Vandenbroucke, J P & Rosendaal, F R (1997) The Leiden Thrombophilia Study (LETS) *Thrombosis and Haemostasis*, **78**, 631–635

Research Committee of the British Thoracic Society (1992) Optimum duration of oral anticoagulation therapy for deep vein thrombosis and pulmonary embolism *Lancet*, **340** 873–876

Rosendaal, F R (1997) Thrombosis in the young epidemiology and risk factors A focus on venous thrombosis *Thrombosis and Haemostasis*, **78**, 1–6